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THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group:	1638
Confirmation No.:	9526
Application No.:	09/486,904
Invention:	SELECTIVE EXPRESSION OF GENES IN PLANTS
Applicant:	Snyder, et al.
Filed:	March 3, 2000
Attorney Docket:	3220-66107

Certificate Under 37 CFR 1.8(a)

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on January 29, 2004

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(Signature)

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APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Appellant appeals from the final rejection dated September 1, 2003, of claims 9 and 20-25, all claims pending in this matter.

REAL PARTY IN INTEREST

The real party in interest is Purdue Research Foundation, the assignee, pursuant to an assignment by the inventors, recorded in the U.S. Patent and Trademark Office at Reel 9771, Frame 0658 on February 19, 1999, for the rights in the parent PCT application, PCT/US98/18416.

RELATED APPEALS AND INTERFERENCES

There are no other pending appeals or interferences related to the present appeal.

STATUS OF CLAIMS

Claims 9 and 20-25, all claims pending in the captioned application, are finally rejected. The final rejection of claims 9 and 20-25 is being appealed. Claims 1-8, 15-19 have been cancelled. Claims 10-14 have been withdrawn as being drawn to a non-elected group.

STATUS OF AMENDMENTS

The application as filed presented claims 1-19. In response to a restriction requirement, claims 1-8 and 15-19 were cancelled, and claim 10-14 were withdrawn by the examiner. By appellants' response mailed November 12, 2001, claim 9 was amended and claims 20-23 were added. By appellants' response mailed April 30, 2002, claims 9 and 20-21 were amended and claim 24 was added. By appellants' response mailed October 11, 2002, claim 9 was amended. By appellants' response mailed May 27, 2003 (resubmitted June 16, 2003), claims 9, 20-21, and 23-24 were amended can claim 25 was added. Claims 9 and 20-25, as they currently stand, are attached as Appendix A.

SUMMARY OF THE INVENTION

The present invention is directed to methods for producing a compound in a transgenic plant. The methods comprise the steps of producing a fertile transgenic plant by introducing into plant cells a DNA construct comprising a promoter, a blocking sequence, and a coding sequence of a structural gene coding for a compound that is detrimental to the plant and is commercially valuable, the blocking sequence being flanked by a pair of directly repeated site-specific recombination sequences and wherein the structural gene is operably linked to the promoter only after the removal of the blocking sequence, and culturing the plant cells to produce the fertile transgenic plant; pollinating the transgenic plant to produce transgenic plants that are homozygous for the DNA construct; crossing the transgenic plant homozygous for the DNA construct with a plant having a DNA sequence comprising a coding region encoding a site-specific recombinase that recognizes the site-specific recombination sequences to produce

an F1 plant or seed; expressing the site-specific recombinase in the F1 plant or seed; expressing the compound; and extracting the compound in economical quantities.

ISSUES

The issues presented by this appeal are:

(a) whether the invention defined in rejected claims 9 and 20-25 is indefinite within the meaning of 35 U.S.C. § 112, second paragraph, for failing to particularly point out and distinctly claim the invention;

(b) whether the invention defined in rejected claims 9 and 20-25 is indefinite within the meaning of 35 U.S.C. § 112, first paragraph, for failing to enable one to make and use the invention commensurate with the scope of the claims; and

(c) whether the invention defined in rejected claims 9 and 20-25 is obvious within the meaning of 35 U.S.C. § 103(a) over Kilby, NJ (1995), Plant Journal 8: 637-652, in view of Odell, et al., U.S. Patent No. 5,658,772, and Kilby, NJ (1993), Trends in Genetics 9: 413-421.

GROUPING OF THE CLAIMS

Claims 9 and 20-25 stand or fall together for all rejections.

ARGUMENTS

Claims 9 and 20-25 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Initially, the Examiner finds that the term "gene" of claim 9 is unclear. According to the Examiner, the term "gene" implies a DNA sequence that exists in nature and includes coding and noncoding regions, as well as regulatory sequences associated with expression. Applicants respectfully submit that the term "gene" may be used to in other ways, for example the portion of the DNA that is transcribed into RNA. (See, e.g., MOLECULAR BIOLOGY OF THE CELL (3rd ed., pg 423)). In a previous effort to advance prosecution and to clarify the claim language, the applicants replaced the term "structural gene" with "coding sequence of a structural gene." Applicants believe that this language is now clear and does not

require further amendment. However, if all other issues are resolved, applicants would not object to the Examiner's suggested language in lines 5 and 7 of claim 9.

The Examiner has also rejected claim 9 because the Examiner finds that the term "economical quantities" is unclear. Applicants have previously submitted that the term "economical quantities" refers to a compound that is produced in sufficient quantities that the value of the extracted compound exceeds the costs associated with standard production and extraction methods. The Examiner now contends that the applicants have failed to define "standard production costs." Applicants submit that while standard production costs would vary from crop to crop, and for extraction of various compounds, and such costs would be readily determinable by a production facility. Applicants respectfully submit that the exact parameters of production costs need not be specified to render this claim definite.

Reconsideration of the rejection of claims 9 and 20-25 under 35 U.S.C. § 112, second paragraph, leading to reversal of the Examiner's rejection and passage of the application to issuance is respectfully requested.

Claims 9 and 20-25 stand rejected under 35 U.S.C. § 112, first paragraph. According to the Examiner, the specification, while being enabling for a method of producing a compound that is not detrimental to the plant, is not enabling for a compound that is detrimental to the plant. According to the Examiner, the state of the art is that one skilled in the art can readily make DNA constructs containing a structural gene encoding a compound, transform these into plants, and express them with a reasonable expectation of success. However, according to the Examiner, expressing a compound detrimental to the plant, such as barnase, is more unpredictable, and that expression of a gene encoding such a detrimental compound must be regulated in order to avoid killing all cells expressing the compound. Further, according to the Examiner, recombinase mediated excision of appropriately flanked DNA sequences is variable and yields chimeric phenotypes having both recombined and unrecombined DNA. The Examiner has previously cited Gidoni, D, et al. (2001) Euphytica 121:145-156, for the proposition that embryonal recombination and germline inheritance of recombined tobacco loci show variable recombination efficiencies, and Vergunst (1998) Nuc. Acids. Res. 26: 2729-

2734, for the unpredictability of using the recombinase system as evidenced by instability of recombinants and phenotypic "escapes." Thus, the Examiner concludes that, without further guidance, it is unpredictable that one skilled in the art would be able to express in a plant a structural gene encoding a compound that is detrimental to the plant, as applicants have provided no guidance on how to eliminate predictably inoperable embodiments.

First of all, the specification of the above-captioned application provides ample proof-of-concept examples. While these examples, as discussed on pages 17-31 of the specification, use compounds that are not detrimental, the examples clearly demonstrate that the claimed methods can be used to control expression of a compound for the purposes of extracting that compound, and it is within the ordinary skill in the art to substitute one coding sequence for another. Yet, the Examiner summarily finds that "expressing structural genes encoding a compound detrimental to the plant, such as barnase, is more unpredictable." However, the Examiner provides no basis for this assertion. Applicants question why it would be more unpredictable to express a detrimental compound in the claimed recombination methods, when such methods provide the regulation of expression needed with a compound such as barnase. Even if it is difficult to control barnase expression, merely because expression of a single very lethal compound, barnase, may be difficult to control, such difficulty does remove expression of the group of detrimental compounds from a person skilled in the art. Additionally, even if a transgenic line were "leaky" and some of the detrimental compound were expressed too early in some plants, such would reduce the yield, and perhaps even kill a percentage of the transgenic plants, but this would not render the invention inoperative. The Examiner simply has not substantiated an enablement rejection based on the difference in the predictability of expression of detrimental verses non-detrimental compounds.

Furthermore, applicants respectfully submit that recent work has demonstrated that site-specific recombinases can be nearly 100% efficient. Attached to the response of May 27, 2003 (resubmitted June 16, 2003) as Exhibit A is Zuo et al. (2001) Nature Biotechnology 19: 157-616. In Zuo, all 19 of the Arabidopsis lines created using a Cre/Lox mediated excision system underwent excision. Luo et al. (2000) The Plant Journal 23(3): 423-430, attached to the response of May 27, 2003 (resubmitted June 16, 2003) as Exhibit B, shows similar success with

FLP/FRT, the site-specific recombinase system of claim 23. Instances where the efficiencies of the recombinases were found to be low can be attributed to weak promoters, or, as discussed by Luo, may be due to position effects (Low at p. 427). Genes may be silenced due to these position effects, thus negating their expression. These deleterious effects may be overcome by selecting the transgenic lines whose transgenes are not silenced. Such selection is within the ordinary skill in the art.

Applicants acknowledge that the Examiner has noted that the Zuo and Luo references were not previously submitted in an Information Disclosure Statement. However, these references are post-filing date references and are not prior art. These references are being submitted only to refute the Examiner's claims of lack of predictability, as evidenced by the Examiner's own post-filing date references. Accordingly, applicants do not believe that an Information Disclosure Statement is required. If an Information Disclosure Statement is required, applicants would be happy to submit one.

The Examiner also rejects the Zuo and Luo references because these reference do not contain the exact method as the instant case. In particular, the Examiner points out that different promoters are used and the recombinase sequences remove different blocking sequences. However, the presently appealed claims do not require a specific promoter and blocking sequence. Furthermore, the applicants' references are at least as similar to the disclosed embodiments than several of the references cited by the Examiner. For example, Luo use FRT/FLP, as in the examples provided in the present specification, whereas the Vergunst reference cited by the Examiner uses the same promoter that the Examiner criticizes in Zuo, 35S CaMV, and uses the Cre/Lox recombination system, rather than FRT/FLP. Thus, the Zuo reference is closer to the disclosed embodiments than that of the Vergunst reference, and the group of references cited by the applicants are at least, if not more, applicable than the group of references cited by the Examiner.

In sum, applicants respectfully submit that the Examiner made improper conclusory statements regarding unpredictability in expressing structural genes encoding compounds in plants and the Examiner improperly relied on post-filing date references to find lack of enablement, while improperly dismissing the applicants' post-filing date references of

equal or better relevance. Applicants respectfully submit that the art is sufficiently predictable to enable one of ordinary skill in the art to make and use the invention. Accordingly, reconsideration of the rejection of claims 9 and 20-25 under 35 U.S.C. § 112, first paragraph, leading to reversal of the Examiner's rejection and passage of the application to issuance is respectfully requested.

Claims 9 and 20-25 remain rejected under 35 U.S.C. § 103(a) as being obvious over Kilby (1995), Plant Journal 8: 637-652, in view of Odell, U.S. Patent No. 5,658,772, and Kilby (1993), Trends in Genetics 9: 413-421. The Examiner finds that the term "extracting the compound in economical quantities" is given no patentable weight due to lack of clarity of this claim term. Accordingly, the Examiner reiterates this prior rejection.

As discussed above, applicants respectfully submit that the term "economical quantities" refers to a compound that is produced in sufficient quantities that the value of the extracted compound exceeds the costs associated with standard production and extraction methods. The Examiner has maintained this rejection because the Examiner finds that this definition "does not teach what 'standard production costs are.'" Applicants submit that standard production costs would vary from crop to crop, and for extraction of various compounds. While applicants do not define standard production costs, such costs should be easily and readily determinable.

Furthermore, even assuming, *arguendo*, that it "would have been *prima facie* obvious and well within the means of one of ordinary skill in the art at the time the invention was made to use the strategy of expressing a biologically detrimental compound only after removal of a blocking sequence," applicants respectfully submit that none of the Kilby or Odell references, alone or in combination, suggest the use of the claimed structures for the expression and extraction of the detrimental compound, and the references certainly do not teach or suggest extraction of economical quantities of the compound. Instead, Odell teaches the use of a construct to produce barnase for *in situ* disruption of the seed development. Odell is silent on the quantity of barnase produced. Indeed, Odell does not even indicate that an extractable amount of barnase would be produced, and certainly not an economical quantity. While Odell

teaches extraction of DNA and RNA for analysis purposes, this is different and clearly distinct from extraction of the resultant barnase protein, which may or may not be present and stable in extractable quantities. Further, because the barnase is used for *in situ* disruption of the seed development to produce seedless watermelon, extraction of the barnase would be counter to the disclosed use of this detrimental compound. Also, that Kilby (1993) suggests the use of a construct for the study of harmful mutations in no way teaches or suggests that a mutation gene product could be expressed and extracted in economic quantities. Kilby (1995) is completely silent regarding detrimental compounds and, thus, does not suggest expression and extraction of such compounds in quantities sufficient for commercial production. Odell, alone or in combination with the Kilby references, simply fails to teach or suggest production of a detrimental compound and extraction of that same compound.

Reconsideration of the rejection of claims 9 and 20-25 under 35 U.S.C. § 103(a) leading to reversal of the Examiner's rejection and passage of the application to issuance is respectfully requested.

Reconsideration of the rejections leading to reversal of the Examiner's rejections and passage of the application to issuance is respectfully requested.

Respectfully submitted,

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Appendix A

9. A method for producing a compound, said method comprising the steps of

producing a fertile transgenic plant by introducing into plant cells a DNA construct comprising a promoter, a blocking sequence, and a coding sequence of a structural gene coding for a compound that is detrimental to the plant and is commercially valuable, said blocking sequence being flanked by a pair of directly repeated site-specific recombination sequences and wherein the structural gene is operably linked to the promoter only after the removal of said blocking sequence, and culturing the plant cells to produce the fertile transgenic plant;

pollinating said transgenic plant to produce transgenic plants that are homozygous for the DNA construct;

crossing said transgenic plant homozygous for the DNA construct with a plant having a DNA sequence comprising a coding region encoding a site-specific recombinase that recognizes said site-specific recombination sequences to produce an F1 plant or seed;

expressing the site-specific recombinase in the F1 plant or seed;

expressing the compound; and

extracting the compound in economical quantities.

20. The method of claim 9 wherein the step of crossing said homozygous transgenic plant with a plant having a DNA sequence comprising a coding region encoding a site-specific recombinase produces an F1 plant or seed that expresses the biologically detrimental compound.

21. The method of claim 20, wherein the extracting step comprises extracting the compound from the plant or seed.

22. The method of claim 9 wherein the promoter is a constitutive promoter.

23. The method of claim 9 wherein the pair of directly repeated site-specific recombination sequences are FRT recombination sequences, and the coding region encoding

the site-specific recombinase encodes the FLP recombinase and is operably linked to a constitutive promoter.

24. The method of claim 9 wherein the step of pollinating said transgenic plant to produce plants that are homozygous for the DNA construct comprises self-pollination.

25. The method of claim 9 wherein the promoter of the DNA construct is a leaf-specific promoter and the extracting step includes extracting the compound from leaves.